IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:	)	Group Art Unit: 1635
BECKER *et al.*	)	Examiner: Shibuya, M.
Serial No. 09/523,237	)	Atty. Docket No. GP068-03.CN1
Filed: March 10, 2000	)	
For: KITS FOR AMPLIFYING TARGET NUCLEIC ACID SEQUENCES USING MODIFIED OLIGONUCLEOTIDES	)	

DECLARATION UNDER 37 C.F.R. § 1.131

Box Non-Fee Amendment
Commissioner for Patents
Washington, D.C. 20231

Sir:

We, Michael M. Becker, Steven T. Brentano and Mehrdad Majlessi, co-inventors of the above-identified patent application, hereby declare as follows:

1. Prior to August 25, 1995, we conceived of and reduced to practice in the United States modified oligonucleotide primers for use in amplifying target nucleic acid sequences, where the modified oligonucleotide primers contained one or more ribonucleotides having a 2'-O-methyl substitution to the ribofuranosyl moiety. Evidence of this prior conception and reduction to practice can be found in attached Exhibit A, which comprises a set of Steven Brentano's laboratory notebook pages setting forth a study which was conducted to test the efficacy of primers containing 2'-O-methyl substitutions in a transcription-mediated amplification procedure. Although the dates on these pages have been redacted, the study set forth therein was completed in the United States prior to August 25, 1995.

2. The amplification study set forth in Exhibit A included primer sets of both T7 and non-T7 oligonucleotide primers. The T7 primers of this study were 50 bases in length,

Considered KAL 04-12-02

possessed the same base sequence (taking into account DNA/RNA equivalents), and differed in their structures only as follows: (i) the "T7ArpoHIV4195(-)" primers contained only unmodified deoxyribonucleotides; (ii) the "T7ArpoHIV4195(-)m13" primers contained 37 unmodified deoxyribonucleotides and 13 2'-O-methyl modified ribonucleotides positioned at the 3' most end of these primers; (iii) the "T7ArpoHIV4195(-)m18" primers contained 32 unmodified deoxyribonucleotides and 18 2'-O-methyl modified ribonucleotides positioned at the 3' most end of these primers; (iv) the "T7ArpoHIV4195(-)r13" primers contained 37 deoxyribonucleotides and 13 unmodified ribonucleotides positioned at the 3' most end of these primers; and (v) the "T7ArpoHIV4195(-)r18" primer contained 32 unmodified deoxyribonucleotides with the 18 unmodified ribonucleotides positioned at the 3' most end of these primers. A single non-T7 primer was used in this study, which is identified as the "HIV4116" non-T7 primer.

3. The primers of this study were all tested under essentially identical amplification conditions and at concentrations of 8, 15 or 30 pmol of the T7 primer and 30 pmol of the non-T7 primer in the presence of $5x10^5$ copies of an HIV target sequence or in the absence of the HIV target sequence. Following the addition of each primer set to an amplification reaction mixture under amplification conditions and for a period of time sufficient to amplify target sequence present in an amplification reaction mixture, a 1μl aliquot of amplification reaction mixture was removed from each 100 μl amplification reaction mixture present in each reaction vessel. The 1 μl aliquots of amplification reaction mixture were then added to separate vessels, each containing 100 μl of deionized water.

4. Amplified target sequence present in the sample of each reaction vessel (either the remaining 99 μl of undiluted amplification reaction mixture or the 101 μl of diluted amplification reaction mixture) was then determined using a homogenous format described as the Hybridization Protection Assay (HPA) in the instant application, (*see* specification at page 5, lines 10-18), and acridinium ester (AE)-labeled probes specific for a target sequence present in the

amplified HIV target sequence. Each sample received 0.1 pmol of AE-labeled probe and 4 pmol of identical cold probe, creating a competition assay as described in the instant application (*see* specification at page 10, lines 19-24). Signal from each sample was measured in relative light units (RLUs) using a luminometer.

5. The results of this study are recorded on page 67 of Exhibit A and are separated into various groupings based on the concentration and structure of the T7 primer tested. Those groups based on the concentration of target sequence present in the amplification mixture are identified as follows: (i) "-" represents the absence of target sequence in the amplification reaction mixture; (ii) "500 copies full format" represents the presence of $5x10^5$ copies of the HIV target sequence in the amplification reaction mixture prior to amplification and without any subsequent dilution; and (iii) "500 copies 1 μl" represents the presence of $5x10^5$ copies of the HIV target sequence in the amplification reaction mixture prior to amplification, with 1μl of the amplification reaction mixture being diluted with 100 μl of deionized water subsequent to amplification and prior to detection. And designations for the T7 primer structures are presented as follows: (i) fully deoxyribonucleotide T7 primers are designated as "N-8", "N-15" and "N-30"; (ii) T7 primers having 13 3' end 2'-O-methyl modified ribonucleotides are designated as "m13-8", "m13-15" and "m13-30"; (iii) T7 primers having 18 3' end 2'-O-methyl modified ribonucleotides are designated as "m18-8", "m18-15" and "m18-30"; (iv) unmodified T7 primers having 13 3' end ribonucleotides, with the remaining bases being deoxyribonucleotides are designated as "r13-8", "r13-15" and "r13-30"; and (v) unmodified T7 primers having 18 3' end ribonucleotides, with the remaining bases being deoxyribonucleotides are designated as "r18-8", "r18-15" and "r18-30". The second number in each case indicates the amount of T7 primer added to the amplification reaction mixture in pmol. All results are presented in terms of (RLUs) detected.

DECLARATION

Serial No. 08/893,300
Atty. Docket No. GP068-02.UT

6. As the results of this study demonstrate, 2'-O-methyl modified primers can be used to successfully amplify target nucleic acid sequences. This is evidenced, for example, by a comparison of results from samples containing no target sequence with samples including either 2'-O-methyl modified primers or unmodified deoxyribonucleotide primers. (It is noted that the excessive RLU value for the "r18-15" sample under the "-" category on page 67 of Exhibit A would suggest that this sample was contaminated with target sequence.) The results for both the 13 and 18 base modified primers in these tests were very similar.

We hereby declare that all statements made herein of our own knowledge are true, and that statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of this application and any patent issuing therefrom.

Date: February 4, 2002 By: _____
Michael M. Becker, Ph.D.

Date: 2-4-02 By: _____
Steven T. Brentano, Ph.D.

Date: 2/4/02 By: _____
Mehrdad Majlessi

EXHIBIT A

TITLE 2'OMe & RNA T7proHIV 4195E) Primer Test

Project No. AMP-T
Book No. 3222

From Page No. X

Purpose: Test T7proHIV4195E) primer w/ either 13 or 18 bases of 2'OMe or RNA on 3' end (see 3222:3 & 2837:57). Use lots of target to start. Compare to normal 4195 primer. Check overall signal & consistency

Procedure:

- amp w/ all three T7 primers at 8, 15, or 30 pmol
- use HIV 4116 as non T7 at 30 pmol

	ref	OD/ml	μg/μl	pmol/μl
[redacted]	mm 3184-33	~~17.8~~	~~0.712~~	~~43.4~~ 36.9
T7AproHIV4195E)m13	mm 3189-42	17.8	0.712	43.4
T7AproHIV4195E)m18	mm 3189-42	19.75	0.79	48.2
T7AproHIV4(95E)r13	mm 3227-8	6.5	0.26	15.86
T7AproHIV4(95E)r18	mm 3227-6	9.9	0.396	24.16

all 50 long

- non-T7 =
[redacted] mm 3097:99 60.1 pmol/μl (3.5 μl HIV aliquot)

- 3⊕ & 3⊖ amps ea primer, so make mix x 7

- target: use 500 copies la amp (15 primer x 3 conc x 3 rep = 45 so make x 60)
 - for 60 of 50 μl x 500 copies = 3x10⁴ copies in 3 ml
 - Target = 2x10⁷ copies/μl ← No! 2x10⁷/μl so used 500,000 copies!
 = 2x10⁴/μl
 → so use 1.5 μl + 3 ml H_2O = 500 copies/50 μl

To Page No. 65

Witnessed & Understood by me, MHBott | Date | Invented by | Recorded by [signature] | Date

Project No. AMP-T
TITLE cont Book No. 3222 65

From Page No 64

- make premix x115 (105 amps) at 25 ul/amp

2875 ul AR (9506061X recon w/ H2O)
57.4 ul #116 (210 pmol for 7 amps = 3.49 ul ea)

- ampmix x7

x7	T7 pmol x1	T7 pmol x7	T7 ul x7	premix
W-8	8 pmol	56 pmol	1.52 ul	175 ul
W-15	15	105	2.85	
W-30	30	210	5.69	
m13-8	8	56	1.29	
m13-15	15	105	2.42	
m13-30	30	210	4.84	
m18-8	8	56	1.16	
m18-15	15	105	2.18	
m18-30	30	210	4.36	
r13-8	8	56	3.53	
r13-15	15	105	6.62	
r13-30	30	210	13.24	
r18-8	8	56	2.32	
r18-15	15	105	4.35	
r18-30	30	210	8.69	

mix w/ primer pool

- Do ampososomal hybl ampl MTD assay

~~MTD~~ general	AR	9506061X
MTD2	ER	9503028X
MTD2	EDB	9505032X

- 25 ul ampmix/tube (3@ 30 ea)
- 50 ul target or H2O
- 60°C 10 min 42°C 5 min
- ~~42°C~~ add 25 ul enz rgt (2000u RT, T7)
- 42°C 90 min

To Page No. 66

Witnessed & Understood by me, [signature] Date
Invented by [signature] Recorded by Date

TITLE cont

Project No. AMP-T
Book No. 3222

From Page No. 65

- HPA 1 µl ē 99 µl } w/ 4 pmol cold crude
  (+100 µl H₂O) 0.1 pmol AE

- probe mix × 160
  16 ml 2× hyb
  16 pmol AE probe = 200 µl of 80 fmol/µl
  640 pmol cold crude = 1.5 µl (at 425 pmol/µl)

  (NO! conc of crude oligo really 545 pmol/µl → really 5 pmol ea)

- hyb 15 min 60°C
- 300 µl sel. OH 10 min 60°C
- H_2O to cool
- read C&TD 2 sec

Results:

- data next page

- ⊖ all pretty high ~10,000 RLU
  - to bleach pipets for next time

- all Normal ē RNA ē 2' OMe primer amps worked pretty well & had saturated signal levels but only

- try again w/ less target

To Page No. 67

Witnessed & Understood by me, [signature] Date

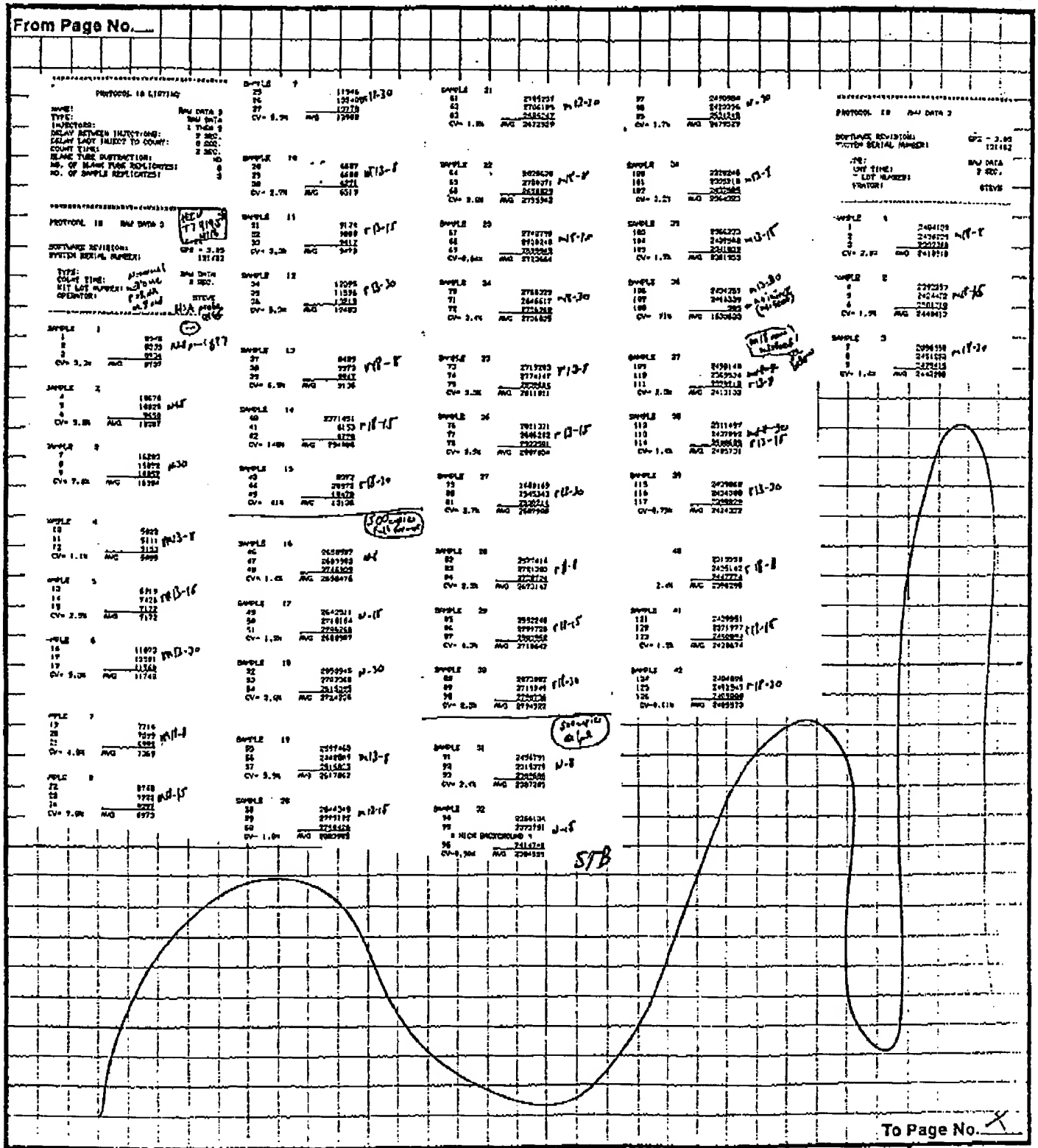
Invented by [signature] Date
Recorded by

TITLE Cont

Project No. AMP-T

Book No. 3222

From Page No.



To Page No. X

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	